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Original Article

Radioprotective Role of Folic Acid on Testicular Structure and Serum Testosterone in Male Rabbits Exposed to X-Ray Irradiation.

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Abstract
Background: Exposure to ionizing radiation, particularly X-rays, induces harmful effects on radiosensitive tissues such as the male reproductive system through the generation of reactive oxygen species, resulting in oxidative stress, DNA damage, and cellular degeneration. These alterations negatively affect spermatogenic cells and Leydig cell function, leading to impaired spermatogenesis and decreased testosterone production. Folic acid, an essential B vitamin involved in DNA synthesis, repair, and antioxidant defense, has been proposed as a potential radioprotective agent. This study aimed to evaluate the radioprotective effects of folic acid on testicular tissue and serum testosterone levels in male rabbits exposed to X-ray irradiation. **Materials and Methods:** Twenty adult male rabbits were randomly assigned to four equal groups (n = 5): control, folic acid–treated (0.07 mg/kg body weight orally for one month), X-ray–exposed (80 kV, distance 98 cm, once daily for one week), and a folic acid plus X-ray group in which animals received folic acid for one month prior to irradiation. Clinical signs and body weight were monitored throughout the experiment. Serum testosterone levels were measured using ELISA, and testicular tissues were collected for histopathological examination following hematoxylin and eosin staining. Statistical analysis was performed using one-way ANOVA with significance set at $P \leq 0.05$. **Results:** X-ray exposure caused a significant reduction in serum testosterone levels and induced marked histopathological alterations, including edema, necrosis of spermatogenic cells, reduced germinal layers, and oligospermia. In contrast, folic acid administration, either alone or prior to irradiation, maintained testosterone levels near normal values and markedly reduced testicular damage. Pretreated rabbits exhibited preservation of seminiferous tubule architecture and improved cellular integrity. **Conclusion:** These findings indicate that folic acid exerts a significant protective effect against X-ray–induced testicular injury and may serve as a supportive strategy to mitigate radiation-induced reproductive toxicity when used within approved therapeutic doses.
Keywords: folic acid , Testicular , X-ray, rabbits , Spermatogenic .

INTRODUCTION

Energy that is transported or radiated in the form of particles or waves is known as radiation. Electromagnetic waves are categorized as either non-ionizing radiation or ionizing radiation based on their energy and frequency. Ionizing radiation possesses sufficient energy to extract electrons from atoms, thereby forming ions. X-rays are a type of electromagnetic radiation that originates outside the atomic nucleus and has lower energy than gamma rays. They are predominantly generated through artificial processes, with their primary use being in medical applications [1]. Ionizing radiation is capable of ionizing atoms by ejecting electrons from their atomic orbits, thereby creating positively charged ions and free negatively charged electrons. This form of radiation arises from both natural sources and human-made radioactive materials [1]. Its qualities can be applied to manufacturing, agriculture, disease diagnostics and treatment, and energy generation [2]. It encompasses high-energy electromagnetic waves such as X-rays and gamma rays which have shorter wavelengths and greater energy compared to ultraviolet or visible light. It also includes various high-energy particles, including electrons, protons, neutrons, and alpha particles. [3]. Ionizing radiations are known to cause oxidative stress on target tissues, primarily by producing reactive oxygen species (ROS), which upset the balance between pro-oxidants and antioxidants in the cells [4]. ROS also attack a variety of cellular macromolecules, including proteins, lipids, and DNA, ultimately leading to the death of cells [5]. Ionizing radiation is utilized in industrial and medical settings, and one type of it is called an X-ray [6]. Chronic alterations in a number of radiosensitive organ systems, such as the kidney, heart, or lung, may occur in survivors, and certain organs, such as the reproductive system [7]. or cataract of the eye lens [8], may exhibit clinically significant alterations even at extremely low dosages. The general features of acute radiation sickness have been described recently in more detail, with special emphasis on animal studies, as this is a rare

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event in humans. Acute radiation syndrome, sometimes known as radiation sickness, is the term used to describe whole-body exposure to high doses of radiation. In addition to disrupting biochemical parameters, this condition can affect the hematopoietic [9]. cardiovascular [10], and digestive systems [11]. Developing brains are susceptible to ionizing radiation, according to several studies [1-12]. Prenatal X-irradiation has been linked to histological alterations in the brain in humans and experimental animals, as well as deficits in learning and memory [13]. Radiation effects depend primarily on the amount of dose absorbed by a specific organ, and the variation in effects between external and internal radiation sources is largely due to how the dose is distributed within and across different organs of the body. [14].

Aim of the work:

This study was designed to evaluate the radioprotective effect of folic acid on the histological structure of testicular tissue in male rabbits exposed to X-ray irradiation, with particular emphasis on spermatogenic cell integrity and seminiferous tubule architecture. In addition, the study aimed to assess changes in serum testosterone levels as a supportive functional parameter to further elucidate the protective role of folic acid.

Material and Methods

Material used:

1.1. Experimental Animals

The study employed twenty adult male rabbits, all native to the area, weighing between 2.00 and 2.32 kilograms. The rabbits were kept in groups in cages of 100 x 85 x 45 centimetres at the Zoology Department's facilities at Omar Al-Mukhtar University. The temperature was set at 25±1˚C, and the photoperiod was maintained at a 12-hour light/12-hour dark cycle. The rabbits were given commercial standard pellets to eat and were given unlimited access to water. One week before to the start of the experiment, the animals were acclimated to the laboratory environment.

Experimental groups and protocol: Rabbits were randomly distributed into four groups (five rabbits/group).

Group I: control - normal control rabbits were given distilled water orally daily.

Group II: Healthy male rabbits were administered folic acid orally at a dose of 0.07 mg/kg body weight daily for a month. The folic acid dose (0.07 mg/kg body weight) was selected based on previous experimental studies demonstrating its antioxidant and cytoprotective effects without inducing toxicity and mentioned a specific dosage used in animal studies (e.g., rabbits or piglets) for research purposes. (This dose has been reported to effectively enhance cellular antioxidant defense and support DNA synthesis and repair mechanisms in experimental models exposed to oxidative stress. [16-15].

Group III: (X-ray exposure group): The X-ray irradiation was performed using a biological X-ray irradiation. Healthy male rabbits were exposed to X-ray irradiation at a dose of 80 kV, from a distance of 98 cm, once daily for one week. [17-18]. The X-ray irradiation was performed using a biological X-ray irradiation apparatus. The model rabbits received whole-body X-ray irradiation at a single dose of 4 Gy (the intensity of 1.2 Gy/min), and the control rabbits received 0 Gy based on the study of Gao et al. [19].

ray irradiation

The X-ray irradiation was performed using a biological X-ray irradiation apparatus (R 000Pro; Rad source, USA)
at the Animal Core Facility of Nanjing Medical University.
The model mice received whole-body X-ray irradiation at a single dose of 4 Gy (the intensity of 1.2 Gy/min), and the control mice received 0 Gy, based on the study of Gao et al. [24]
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The model mice received whole-body X-ray irradiation at a single dose of 4 Gy (the intensity of 1.2 Gy/min), and the control mice received 0 Gy, based on the study of Gao et al. [24]

Group IV: Healthy male rabbits given folic acid at does of 0.07 mg/Kg of body weight daily for a month [20-16]. then exposed to X-ray irradiation at a dose 80kv from a distance of 98 cm once a day for a week [21-22]. All groups of rabbits were experimented on for a month and subject to:

Morphological and clinical signs study:

Every day, the animals were examined to record any behavioral changes, as well as external indicators such as dehydration, eye color, body hair, thick stool, breathing difficulties, bone loss, fever, muscle weakness, activity, and signs of poisoning or death. In addition, the body weight of the rabbits in all groups was measured using electronic balances at the beginning and end of the experiment, and body weight change (%) and weight gain were calculated [23].

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The Blood parameters:
Collect blood samples to determine the following parameters:
Testosterone: - Based on the concept of competitive binding, the IBL-America Testosterone ELISA Kit (Immuno-Biological Laboratories, Inc.) is a solid-phase enzyme-linked immunosorbent test (ELISA). An antibody that targets a specific antigenic location on the testosterone molecule is coated onto the microtiter wells. When it comes to binding to the coated antibody, endogenous testosterone in a sample faces competition from a testosterone horseradish peroxidase conjugate. Washing out the unbound conjugate occurs after incubation. The amount of bound peroxidase conjugate and the sample's testosterone concentration are inversely correlated. Following the addition of the substrate solution, the patient sample's testosterone concentration causes the intensity of colour to reverse [24-25].
Histopathological studies: Upon completion of the trial, the animals were euthanized by cervical dislocation, and postmortem examinations were performed on each animal to identify any unusual or noticeable alterations in internal organs. All animals had their testicles meticulously removed. The tissues were immediately dehydrated through a graded series of ethyl alcohol and then immersed in 10% formalin for 24 hours, a standard histopathological procedure that preserves cellular morphology and prevents autolysis and putrefaction [26]. The samples underwent three paraffin impregnations before being immersed in paraffin wax, which has a melting point of 56°C to 60 °C. Sections of 5–7 µm were cut from the paraffin blocks using a Leica RM 2125 rotary microtome. The deparaffinized sections were stained with Harris' haematoxylin and eosin (H&E) according to Bancroft and Gamble [27], then mounted with Canada balsam and cover slides [28]. A light microscope equipped with a digital camera (Nikon Eclipse E400) was used to evaluate histological sections, and histological alterations were identified and captured on video.
Statistical Analysis:- The statistical analysis was to do used is GraphPad Prism version 4.00, GraphPad software, San Diego, USA. To demonstrate the statistical significance between the group means, a one-way ANOVA with Tukey's HSD test was run. The findings were presented in terms of mean ± Standard error of the mean (SEM). A statistically significant P-value was defined as one that was less than 0.05.

Results:
Result of morphological and clinical signs study: -
From daily observation, there were no obvious abnormal signs in behavioral or external features including body fur condition and feces consistency in any of the treated rabbits compared with all other groups. This general clinical stability is reflected in the body weight data presented in Table 1, where all groups showed only minor fluctuations between initial and final measurements. The control and X-ray groups maintained nearly constant body weights, indicating that the radiation dose administered did not induce marked systemic stress capable of causing weight loss. Rabbits receiving folic acid alone demonstrated a slight increase in body weight, which may suggest a supportive role of folic acid in maintaining normal metabolic activity. Meanwhile, the group treated with both folic acid and X-ray irradiation preserved their body weight close to baseline levels, implying a potential protective effect of folic acid in mitigating any subtle radiation-related physiological changes. Overall, the stability in body weight across all groups aligns with the clinical observations and confirms the absence of acute toxicity throughout the experimental period.

Table 1: The possible Radio-protector effect of folic acid on body weight in male rabbits exposed to X-ray (M ± SE)

Group \ Time	Mean of initial of body weight (gm)	Mean of final of body weight (gm)
Control group	2.12 ± 0.12	2.10 ± 0.11
Folic Acid group	2.00 ± 0.15	2.20 ± 0.21
X-ray group	2.05 ± 0.13	2.10 ± 0.13
Folic Acid and X-ray group	2.25 ± 0.13	2.20 ± 0.13
$p \leq 0.05$, M= Mean ,SE= Stander Error		

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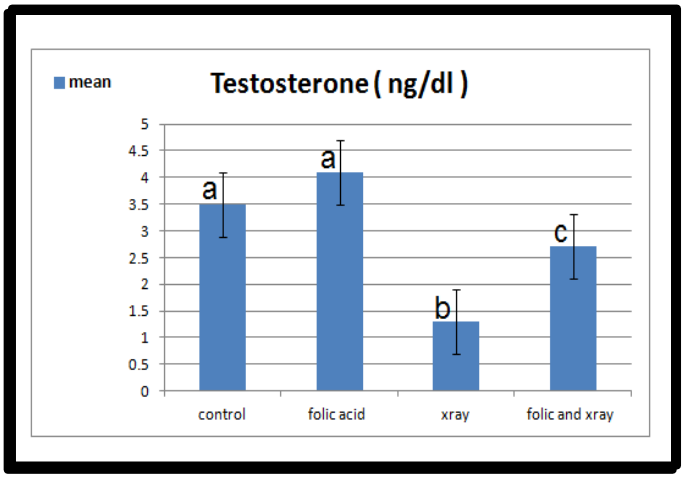
Table 2; Figure 1 shows the effect of folic acid on testosterone levels in male rabbits exposed to X-ray irradiation. The results indicate that the X-ray group experienced a marked and significant decrease in testosterone concentration (1.3 ± 0.1 ng/dl) compared with the control group (3.5 ± 0.6 ng/dl), reflecting the known suppressive impact of ionizing radiation on testicular endocrine function. In contrast, rabbits receiving folic acid alone exhibited a higher testosterone level (4.1 ± 0.3 ng/dl) relative to the control, suggesting that folic acid may enhance or support normal steroidogenesis. Importantly, the group pretreated with folic acid before irradiation showed a substantial improvement in testosterone concentration (2.7 ± 0.1 ng/dl) compared with the X-ray only group. This partial restoration indicates a significant radioprotective effect of folic acid, likely through its antioxidant properties and its role in DNA synthesis and cellular repair.

Table 2: represents the possible Radio-protector effect of folic acid on some physiological parameters in male rabbits exposed to X-ray (M ± SE) :

Parameters	Control	Folic acid	X-RAY	Folic acid & X-RAY
Testosterone (ng /dl)	3.5 ± 0.6	4.1 ± 0.3	1.3 ± 0.1	2.7 ± 0.1
p ≤ 0.05 , M= Mean ,SE= Stander Error				



Figuer. 1: represents the possible Radio-protector effect of folic acid on testosterone (ng /dl) in male rabbits exposed to X-rays. The possible Radio-protector effect of folic acid on the Testis section in male rabbits exposed to X-ray:

Figure 26-29 represents the possible Radio-protector effect of folic acid on the testis section in male rabbits exposed to X-ray in the control group, folic acid group, X-ray group & (X-ray & folic acid group) respectively

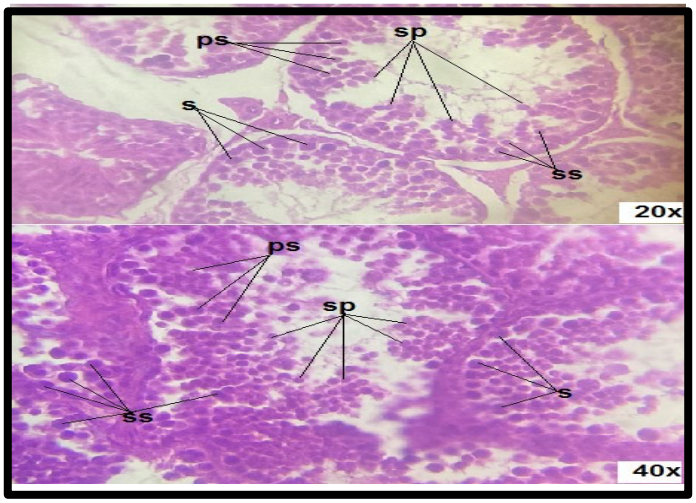


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Figure 2): Photomicrograph illustrates the testis sections of local rabbits showing spermatogonia, primary and secondary spermatocytes, and spermatids.(Control group). (H.E) Sp= Spermatids , S= Spermatogonia , PS= Primary spermatocytes and SS= Secondary spermatocytes

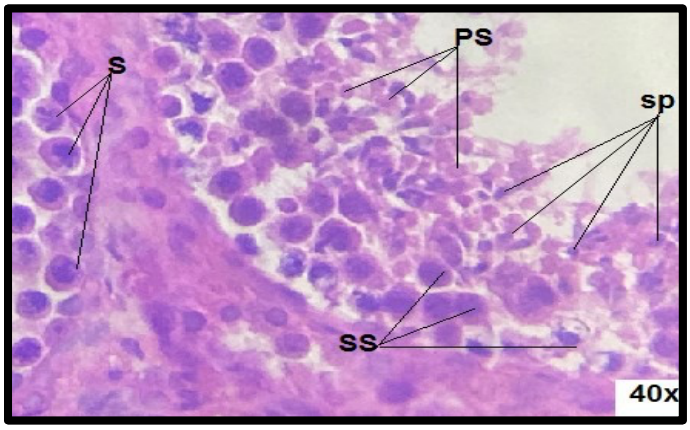

Figure.3: Photomicrograph illustrates testis sections of local rabbits exposed to the folic acid group showing spermatogonia, primary and secondary spermatocytes and spermatids (H.E) S= Spermatogonia , PS= Primary spermatocytes, SS= Secondary spermatocytes and Sp= Spermatids

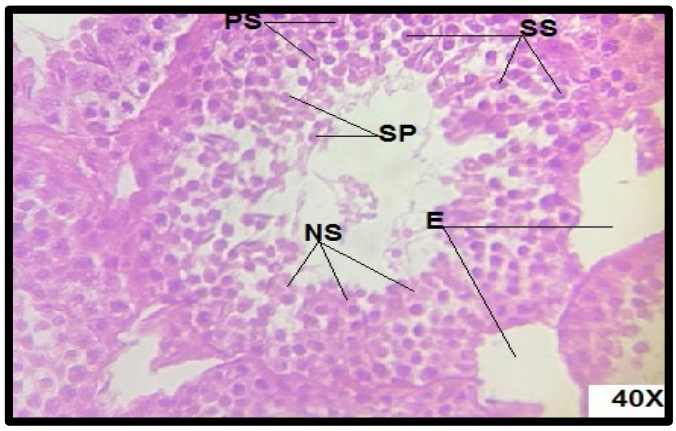

Figure 4:Photomicrograph illustrates testis sections of a local rabbit exposed to the X-ray group. There are clear areas of edema and necrotizing primary and secondary spermatocytes and oligospermia (H.E). NS= Necrotizing Spermatocytes, PS= Primary spermatocytes , E= Edema Sp= Spermatids, and SS= Secondary spermatocytes Oligospermia means a low sperm count

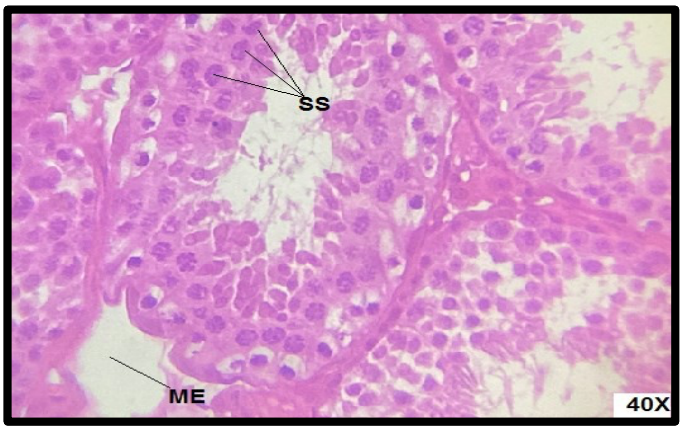

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Figure 5: A Photomicrograph illustrates testis sections of local rabbits exposed to folic acid and X-ray group. There are mild, clear (edematous) areas and decreased secondary spermatocytes. (H.E) SS= Secondary spermatocytes and ME= Mild Ed

Discussion

This study was conducted to evaluate the possible radioprotective role of folic acid on certain physiological indicators and the histological structure of the testes in male rabbits subjected to X-ray exposure. The findings demonstrated that folic acid supplementation provided a noticeable level of protection against radiation-induced biochemical alterations and tissue damage. Continuous daily clinical assessments showed no abnormal physical or behavioral changes in any experimental group, including those exposed to X-ray irradiation.This agrees with previous reports indicating that certain doses of ionizing radiation do not always induce immediate observable behavioral abnormalities [29]. For example, guinea pigs exposed to 15 Gy of whole-body γ-irradiation exhibited no acute behavioral changes, and mice exposed to 8 Gy or 15 Gy also showed no signs of mortality or major neurologic impairment within the first 24 hours post-irradiation [30]. These findings collectively suggest that early behavioral indicators are not always sensitive markers of radiation-induced stress, especially at sublethal or short-term exposure levels. Nevertheless, other research demonstrates that radiation may produce behavioral or neurological alterations depending on the developmental timing and type of tissue irradiated. Prenatal exposure to ionizing radiation (1.5 Gy X-ray) has been shown to impair hippocampal cholinergic neuronal function, resulting in memory deficits and abnormal locomotor activity in adult mice [31-32]. These discrepancies underscore the importance of exposure timing, tissue sensitivity, and dose in determining the clinical manifestations of radiation effects. In the present study, the physiological parameter most impacted by X-ray exposure was testosterone level. Rabbits exposed to radiation demonstrated a marked reduction in serum testosterone, which is consistent with published data describing the susceptibility of Leydig cells to oxidative stress and DNA damage induced by ionizing radiation. Radiation generates reactive oxygen species capable of impairing steroidogenesis, leading to decreased androgen production. However, pretreatment with folic acid significantly improved testosterone levels, supporting its proposed antioxidant and cytoprotective roles. Folic acid contributes to DNA synthesis, methylation reactions, and free-radical neutralization, and has been shown in several studies to mitigate oxidative injury in reproductive tissues. Histological examination further supported these biochemical findings. Testicular sections from the X-ray group showed pronounced structural degeneration, including disrupted seminiferous tubules, reduced germ cell layers, and diminished spermatogenic activity changes also reported in earlier work where radiation induced hemorrhage and cellular damage in multiple organs including testes, liver, lung, and intestine Similarly to [33], documented severe hepatic injury, sinusoidal hemorrhage, and hepatocyte degeneration in rats exposed to 8 Gy abdominal irradiation. Such evidence reinforces the broad spectrum of tissue vulnerability to radiation-induced injury[34].

In contrast, rabbits pretreated with folic acid in the present study exhibited markedly improved testicular architecture, with preserved seminiferous tubule organization and enhanced spermatogenic activity. [35-36].

Conclusion

The present study concludes that folic acid exhibits a clear protective effect on both physiological parameters and testicular histology in male rabbits exposed to X-ray irradiation. Its ability to preserve hormonal balance and tissue structure highlights its value as an effective radioprotective agent. Based on these findings, it is recommended that individuals working in radiation-related medical fields consider folic acid supplementation within medically approved doses, as part of strategies aimed at reducing radiation-induced biological damage.

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